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The surveillance programme for *Aphanomyces astaci* in Norway 2020

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Content

Summary

In this surveillance programme, environmental DNA (eDNA) monitoring is used for the detection of species specific DNA from spores of *Aphanomyces astaci* directly from water filtrates. The presence/absence of eDNA from noble crayfish (*Astacus astacus)* and signal crayfish (*Pacifastacus leniusculus*) is also determined to supplement the results, and to evaluate the habitat status with higher precision. These analyses are part of the collaboration and coordination with the national surveillance programme for noble crayfish. The main geographic focus of this surveillance programme was on the Halden watercourse and neighbouring risk areas. Other covered geographic areas include the Mosse watercourse, Glomma watercourse, and selected areas in the Eidskog municipality including the Buåa watercourse, the Vrangselva watercourse, and River Finnsrudelva. Detection of noble crayfish eDNA, combined with the absence of eDNA from *A. astaci* and signal crayfish, substantiate the presence of non-infected noble crayfish, which is the desired habitat status.

In total, 59, 48, 21 and 36 water samples were collected from selected sites in the Halden-, Mosse-, Glomma watercourse regions and in the Eidskog region, respectively. Locations for sampling water were strategically selected and focused on both control zones and the risk areas adjacent to crayfish plague control zones. The presence/absence of the three target species was determined simultaneously through screening with species-specific qPCR assays.

In 2020, eDNA of *A. astaci* was only detected in the southern part of Lake Rødnessjøen, and no spread of the pathogen was observed in any of the monitored areas.

- In the control zone of the Halden watercourse, *A. astaci* eDNA was detected in the southern part of Lake Rødenessjøen. Here, the known presence of signal crayfish was also confirmed by positive eDNA detection. Within the control zone, *A. astaci* was not detected in any other of the stations. All water samples in the risk area of the Halden watercourse region were negative for eDNA from *A. astaci* and signal crayfish, while most samples were positive for noble crayfish eDNA.
- In the Mosse watercourse, no eDNA of *A. astaci* or signal crayfish was detected, while noble crayfish eDNA was detected upstream of Lake Langen and in the River Tangenelva.
- In the Glomma watercourse, there were no detection of eDNA from noble crayfish, nor from signal crayfish or *A. astaci*.
- In Eidskog municipality, all samples were negative for both signal crayfish and *A. astaci*, while several samples were positive for noble crayfish eDNA in River Vrangselva and River Finnsrudelva.

In summary, eDNA from *A. astaci* was not detected anywhere else than from the signal crayfish location in Lake Rødnessjøen. Frequent detections of noble crayfish eDNA within the regulated *A. astaci* control zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggests the presence of vital noble crayfish populations within *A. astaci* regulated and restricted zones. This actualize a coordinated plan for defining criteria for documentation of infection freedom.

Introduction

The oomycete *Aphanomyces astaci*, the causative agent of the crayfish plague, is a lethal pathogen to native European freshwater crayfish (1-3). It is carried and transmitted by North American freshwater crayfish, which act as healthy carriers of the pathogen. *A. astaci* reproduces and spreads with swimming zoospores, the infective stage of the pathogen. It was accidentally introduced to Europe in the 1860s, and resulted in mass-mortalities of freshwater crayfish all over Europe. It was later re-introduced through many independent introductions of alien North American carrier crayfish (3), in particular signal crayfish.

Crayfish plague is a list 3 disease in Norway, according to the "*Regulation on animal health requirements for aquaculture animals and products thereof, prevention and control of infectious diseases in aquatic animals*" FOR 2008-06-17-819.

Since 1971, seven water systems in Norway have been affected by crayfish plague outbreaks one or several times (4-5). These include the Vrangselva watercourse and River Veksa (1971), the Glomma watercourse (1987 and 2003), Lake Store Le (1989), the Halden watercourse (1989, 2005 and 2014), River Lysakerelva (1998), Buåa watercourse (2010), and Mosse watercourse (2016). In 2016, crayfish plague was confirmed in noble crayfish inhabiting the bordering watercourse Vrangselva and River Billa between Norway and Sweden (which is called River Finnsrudelva on the Norwegian side), but the infection has not yet reached the Norwegian side. In addition, four more localities have been (or still are) subject to crayfish plague regulations due to illegally introduced and confirmed *A. astaci* positive signal crayfish (4). These include Dammane (Vestfold and Telemark), Ostøya (Viken), The Fjelna watercourse (Trøndelag) and Lake Kvesjøen (Trøndelag), where signal crayfish were discovered in 2006, 2009, 2011 and 2013, respectively (4-6). At two of these locations (Dammane and Ostøya), signal crayfish have been successfully eradicated and the areas were declared disease free after several years of surveillance (4).

The focus areas of the 2020 surveillance programme for crayfish plague cover the

- Halden watercourse (under regulation FOR-2015-05-26-592)
- Mosse watercourse (under regulation FOR-2016-12-13-1523)
- Glomma watercourse (under regulation FOR-2005-06-20-652)
- Eidskog municipality, including Buåa watercourse, Vrangselva watercourse and River Finnsrudelva (under regulation FOR-2016-08-17-972)

The Halden watercourse was first struck by crayfish plague in 1989, re-stocked with noble crayfish in the 1990s and the population successfully recovered until the crayfish plague returned in 2005 (7). Immediate closure of the Ørje locks prevented upstream spread. Illegally introduced *A. astaci* positive signal crayfish were found in Lake Øymarksjøen in 2008 (8), leading to the permanent closure of the locks. This prevented further spread, until illegally introduced signal crayfish were found upstream of the locks in 2014. The re-established noble crayfish population in Lake Rødenessjøen was lost during the following plague outbreak (9). In this period, the TARGET project (NRC- 243907) compared cage-based surveillance with environmental DNA (eDNA) monitoring as described in Strand et al. (9). The infection front was followed through analysis of water, and eDNA of *A. astaci* was sometimes detected in the water samples prior to crayfish mortalities in the cages. Noble crayfish and signal crayfish eDNA were also detected in the locations where the crayfish are known to occur (9). After the main

outbreak in Rødnessjøen and the spread of crayfish plague to the River Hølandselva in 2015, *A. astaci* was detected at the outlet of the river in 2016 and in 2019, but not further upstream in the water course (6, 10). Noble crayfish eDNA has been detected from the middle of Hølandselva and upstream from 2016-2019 (6, 10-12; Figure 1)

The Mosse watercourse was struck by crayfish plague in 2016 (13). When the crayfish season started in August, the Norwegian Food Safety Authority (NFSA) received reports regarding possible absence of noble crayfish from Lake Mjærvann and River Hobølelva. No dead crayfish could be found, but eDNA-analyses of water from the small River Tangenelva upstream of Lake Mjærvann (Enebakk) conducted at the Norwegian Veterinary Institute (NVI) confirmed high levels of *A. astaci* eDNA, corresponding to an outbreak situation (13). The NFSA established zone regulations and initiated surveillance with cages in infected areas. In the cage upstream of the lower dam in the pond Steinkistedammen, the spread of crayfish plague was detected in December 2016, while the cage placed in Lake Våg was not affected in 2016 (11). No *A. astaci* eDNA was detected in the Mosse watercourse in 2017, but there was a significant drop in eDNA detection of noble crayfish from June to August in Lake Våg (11). A dead crayfish found in Lake Langen in 2018 where diagnosed with crayfish plague, confirming the upstream spread of crayfish plague in the watercourse (12). No *A. astaci* was detected in the watercourse in 2019 (10).

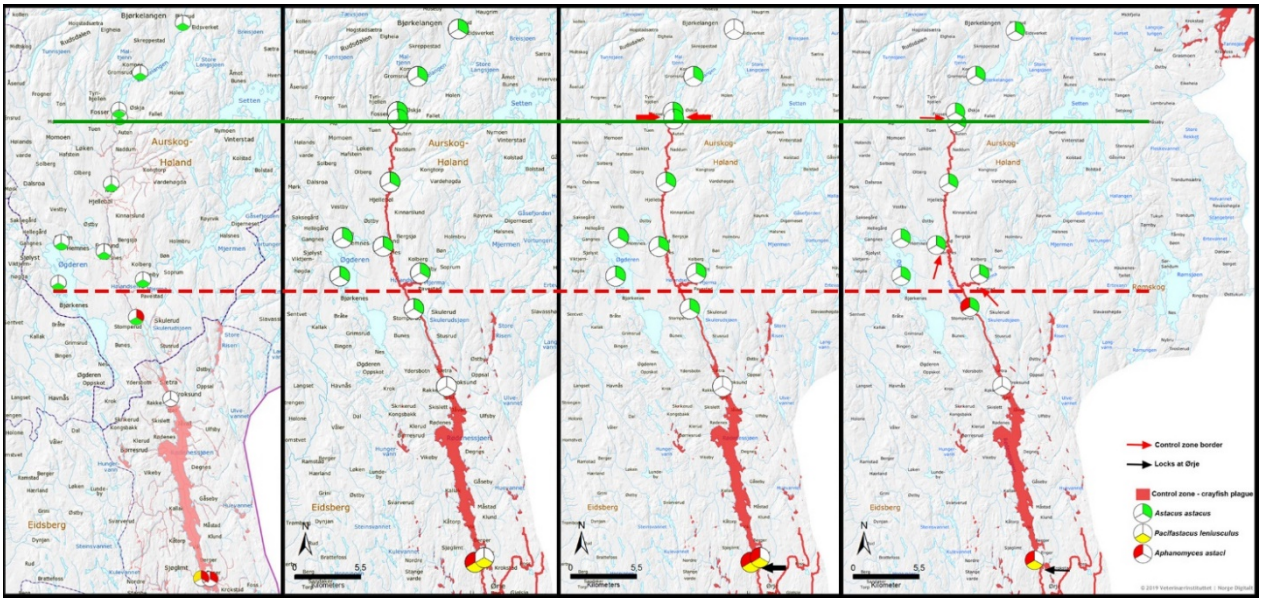

***Figure 1**: Recurring maps of the years 2016 – 2019, showing the stable detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of river Hølandselva (stippled red line) up to the boarder of the control zone at Fosser dam (solid green line).*

The Glomma watercourse was struck by crayfish plague in July 1987, from Kirkenær in Solør and further downstream including Lake Vingersjøen and Lake Storsjøen/River Oppstadåa (4). Environment authorities and landowners cooperated to re-establish crayfish in the river system, but the plague struck again in 2003. Cage experiments combined with crayfish plague diagnostics confirmed active crayfish plague in the system from 2005 until 2015 (4-7). The last detection was in the tributary Opstadåa in 2015. No *A. astaci* eDNA has been detected in the Glomma watercourse, the outlet of Lake Vingersjøen or Oppstadåa in the period 2016-2019 (6, 10-12),

while noble crayfish eDNA was detected in River Oppstadåa in 2016 (6) and at Skarnes and Kongsvinger in 2019 (10).

The Buåa system was struck by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river. A barrier built to prevent the spread of signal crayfish did not stop the infection from spreading, but hopefully stopped the signal crayfish (4). Cage experiments were conducted in the area until 2016 without revealing any active infection source (6). eDNA analysis of samples for Buåa tested negative for *A. astaci* and signal crayfish in 2017, 2018 and 2019 (10-12).

The rivers Vrangselva and Finnsrudelva/Billa in Eidskog municipality, that flow across the border into Sweden were struck by crayfish plague on the Swedish side of the border in 2016. The crayfish plague has been active and slowly migrating upstream in River Finnsrudelva/Billa on the Swedish side of the border in 2017 and 2018. However, no sign of crayfish plague has been detected on the Norwegian side of the border in either of these two watercourses in 2016, 2017, 2018 or 2019 (6, 10-12).

The surveillance programme for *A. astaci* is commissioned by NFSA and conducted by NVI. Until 2015, surveillance of crayfish plague relied on cage experiments with live noble crayfish. In 2016, classical cage experiments was combined with eDNA monitoring (6). Based on an overall assessment taking crayfish welfare and cost-benefit into account, the cage experiments were excluded from the surveillance programme in 2017 (11). From 2018, the program has collaborated with the National surveillance programme for noble crayfish (*Astacus astacus*), commissioned by the Norwegian Environment Agency (NEA) and coordinated by the Norwegian Institute of Nature Research (NINA). This involves joint field work and joint exploitation of water samples and molecular results in overlapping surveillance areas. These synergies enable analyses of a slightly larger sample size than the NFSA-programme alone would allow.

Aims

This surveillance programme aims to

- Monitor the infection pressure and spread of the crayfish plague pathogen *A. astaci* in zone regulated areas as a result of earlier detection of disease (referred to as control zones).
- Substantiate disease free waterbodies in neighbouring areas of the control zones (= risk areas).
- Alert the authorities of any eventual spread of the disease from control zone to risk areas.
- Continue to evaluate eDNA as a monitoring tool for *A. astaci* - alone and in combination with complementary eDNA targets including both the carrier and susceptible crayfish host species.

Materials and methods

Work plan

The surveillance programme is based on eDNA monitoring of water, where DNA from spores of *A. astaci* are detected directly from water filtrates. To complement information on the habitat status, eDNA from the native and susceptible noble crayfish *A. astacus* and the alien carrier signal crayfish *P. leniusc*ulus is monitored within the same water samples. The logistics and analyses are conducted in collaboration with the national surveillance of noble crayfish, funded by NEA, and coordinated by NINA (Figure 2).

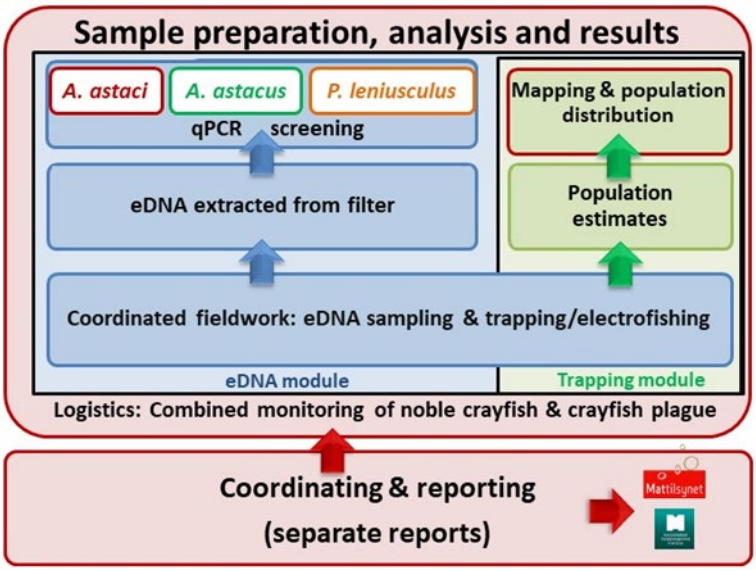

***Figure 2**: Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with the national surveillance of noble crayfish (Funded by the Norwegian Environment Agency (NEA).*

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse, the Glomma watercourse, and Eidskog municipality including the Vrangselva watercourse, Buåa watercourse and River Finnsrudelva. Plotted locations for water sampling, in total 36 sites, as well as the crayfish plague zones, are displayed in Figure 3. Supplementary details are summarised in Appendix 1 (Table S2-S5).

Halden watercourse: The control zone was monitored at a total of 6 sites from Lake Fossersjøen to the south of Lake Rødenessjøen (Ysterud). From previous years monitoring, detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of river Hølandselva (Figure 1) suggests that the upper parts of the system so far has escaped an outbreak. Crayfish localities adjoining the control zone or in close geographical proximity are nevertheless vulnerable to further spread and referred to as "risk zones" (Table S2, Appendix 1). In total, 7 sites were monitored in the risk zone.

Mosse watercourse: The control zone was monitored from Lake Sværsvann and Lake Bindingsvann and downstream to River Hobølelva, in total 10 sites (Table S3, Appendix 1).

Glomma watercourse: The control zone comprises the main passageway downstream Braskereidfoss in Våler. Five sites within the control zone was monitored. (Table S4, Appendix 1).

Eidskog: The control zone (defined by the municipality border) was monitored in the Vrangselva watercourse (4 sites), Buåa watercourse (2 sites) and River Finnsrudelva (2 sites) (Table S5, Appendix 1).

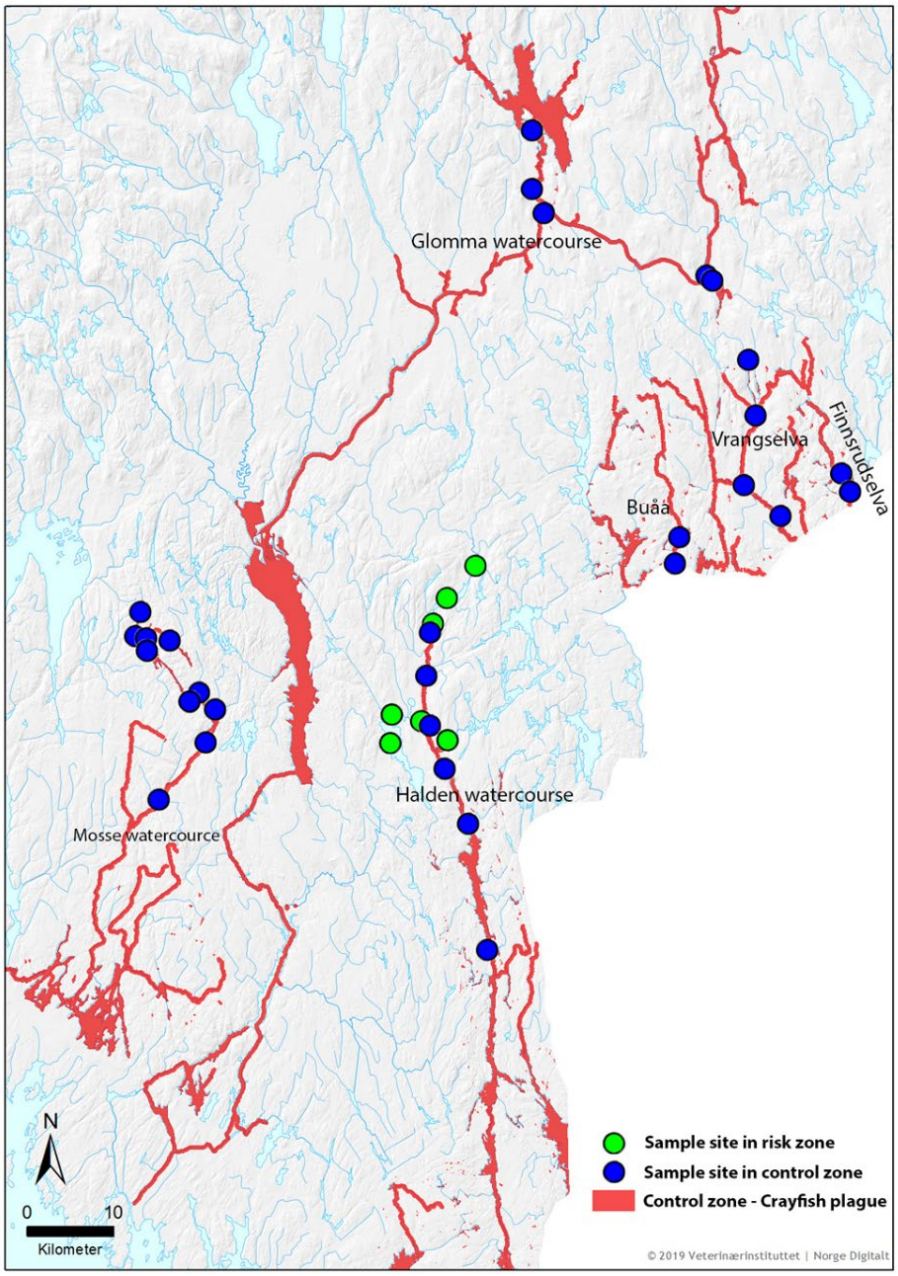

***Figure 3**: Surveillance sites in Eastern Norway 2020. Water samples (circles) were collected in June and August. Regulated areas (crayfish plague control zones) are marked in red. Note: For Glomma, the control zone is an approximation*

eDNA monitoring

The water samples were collected in June and August 2020. From each site, two samples of ~5 L were filtered through sterile glass fibre filters on-site (9). Ideally, 5 L was filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower. In some of these cases extra samples were included to partly compensate for the reduced water volume. This explains the increased number of samples at some sites (Table S2-S5) compared to the agreed number of samples (Table S1).

The filters were transferred with a clean forceps to a ziplock bag containing silica-gel beads or a 15 ml falcon tube with buffer. DNA was extracted from samples collected in June using a CTAB protocol (9) while the a CTAB protocol or NucleoSpin Plant II Midi kit (Marcherey-Nagel) protocol (14). The NucleoSpin Plant II Midi kit protocol yields higher DNA output from the glass fibre filters (14) and the protocol was implemented at the Norwegian veterinary institute during the autumn of 2020.

The extracted DNA samples were screened by qPCR for three DNA targets: the species-specific qPCR assay for *A. astaci* (9, 15) and two crayfish species specific qPCR assays for noble crayfish and signal crayfish developed by Rusch et al. (16). Figure 4 presents an overview of the eDNA monitoring procedure.

***Figure 4**: Water samples of ~5 L each were filtered on-site through glass fibre filters using a portable peristaltic pump (Masterflex E/S portable sampler). Each filter was carefully transferred to a ziplock bag containing silica-gel beads for preservation or a 15ml tube with buffer, until transportation to the laboratory. DNA was isolated with a large volume extraction procedure and presence/absence of eDNA from all target organisms was analysed using qPCR. Figure modified from Vrålstad et al (6).*

Result and Discussion

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 57 water samples representing a total of ~204 L water were analysed. In the control zone, *A. astaci* eDNA was detected in two water samples (in June) at the Southern part of lake Rødenessjøen (Figure 5, Table S2) where signal crayfish were confirmed to be present by positive eDNA results in a total of four water samples (two in June, two in August; Figure 5, Table S2). eDNA from *A. astaci* was not detected at any other station in the watercourse, nor at the outlet of River Hølandselva where it was detected in 2019.The positive detections of noble crayfish eDNA in samples from River Hølandselva and upstream (within the control zone), as observed in the previous years (Figure 1), support the presence of live noble crayfish inhabiting the northern part of the Halden watercourse control zone. This area has apparently escaped the crayfish plague outbreak for the 5th consecutive year. In total, noble crayfish eDNA was detected in 15 water samples from River Hølandselva and upstream (control zone).

All water samples from the risk area surrounding the Halden watercourse were negative for *A. astaci* and signal crayfish eDNA, while most samples, in total 27, were positive for noble crayfish eDNA (Figure 5, Table S2). The combined absence of *A. astaci* eDNA and presence of noble crayfish eDNA suggests that there has been no further spread of the disease in the surveillance period, and that there are live noble crayfish at the monitored sites. This was supported by CPUE (catch per unit effort) data from the national surveillance programme for noble crayfish 2020 (17), where live noble crayfish were documented in Lake Hemnessjøen at a density of 3.72 crayfish per trap-night (CPUE).

eDNA monitoring in the Mosse watercourse

In the Mosse watercourse, 48 water samples representing a total of ~149 L water were analysed. None of the analysed samples showed any sign of *A. astaci* or signal crayfish eDNA (Figure 6, Table S3). eDNA from noble cayfish was detected in ten samples (seven in June and three in August) at two stations upstream Lake Langen. One samples was as also positive for noble crayfish in the River Tangenelva in June. This suggests that crayfish plague has not spread upstream from Lake Langen where crayfish plague was confirmed in 2018, after positive detection on one dead crayfish found at Kilevika (12).

eDNA monitoring in the Glomma watercourse

In the Glomma watercourse, 21 water samples representing a total of ~88 L water were analysed. No sign of *A. astaci* or signal crayfish was found through eDNA analysis (Figure 7, Table S4). No samples were positive for noble crayfish eDNA The results cannot verify any active *A. astaci* infection or infection source from the monitored sites in the Glomma. In September 2020 signal crayfish was discovered in Glomma during a crayfish survey (18) and these were confirmed carriers of *A. astaci* (19). These signal crayfish were captured in traps downstream Solbergfoss, which is in the lower part of Glomma and over 80 km downstream from the eDNA stations in Glomma. The monitored area of Glomma should there be reconsidered.

eDNA monitoring in Eidskog municipality

In the Eidskog municipality, 37 water samples representing a total of ~134 L water were analysed. None of the analysed samples showed any sign of *A. astaci* or signal crayfish (Figure 7, Table S5). In the Vrangselva watercourse, eight samples from Åbogen to Skotterud were positive for noble crayfish eDNA (four in June, four in August), suggesting that the river stretch is still inhabited by live noble crayfish. In River Finnsrudelva, eight samples were positive for noble crayfish eDNA (four in June, four in August).

In the Buåa watercourse, no samples were positive for noble crayfish eDNA and no sign of *A. astaci* or signal crayfish was found through eDNA analysis (Figure 7, Table S5). The Buåa watercourse has been monitored by cages for more than six years (6) and by eDNA alone for another four years. Lack of crayfish plague detection could indicate disease free status. However, a new crayfish plague regulation from August 2016 covers the whole Eidskog municipality (FOR-2016-08-17-972), and replaces the old regulation for the Buåa watercourse. Thus, as long as the Eidskog region is covered by one regulation, no conclusion can yet be drawn regarding disease freedom in the Buåa watercourse.

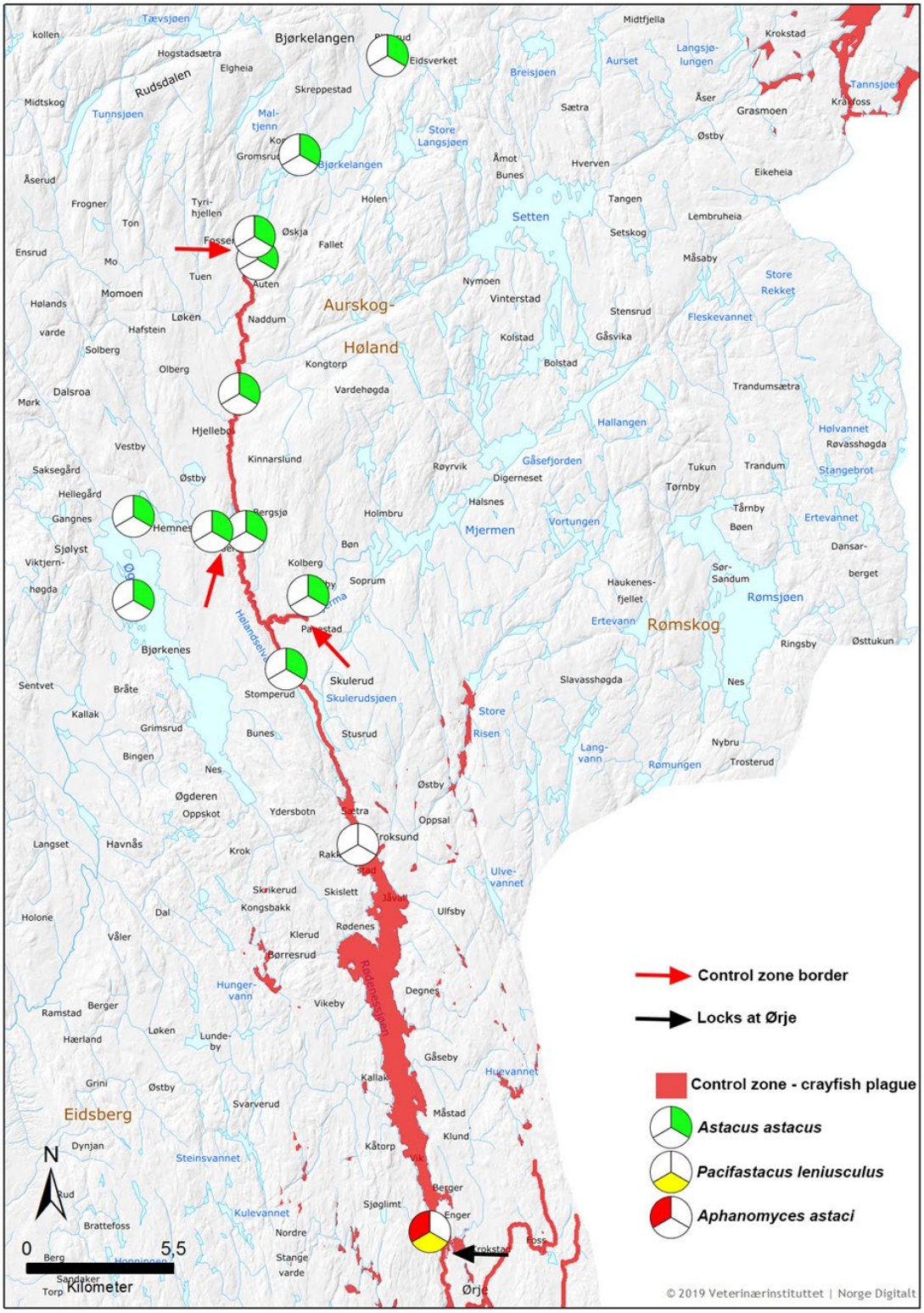

***Figure 5**: Overview map of the surveyed part of the Halden watercourse region in 2020, starting from the Ørje locks (black arrow) in the south where signal crayfish is present. The control area is indicated by red colour on involved lakes and rivers, and ends at Fosserdam (red arrows), which is an artificial barrier for further spread. The pie chart indicates presence (colour) or absence (white) of A. astaci (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. Positive A. astaci samples were detected close to Ørje locks together with* P. leniusculus *eDNA. Only eDNA of noble crayfish was detected in the water upstream the outlet of River Hølandselva. The same was observed in the risk area, suggesting no spread of A. astaci in the monitoring period.*

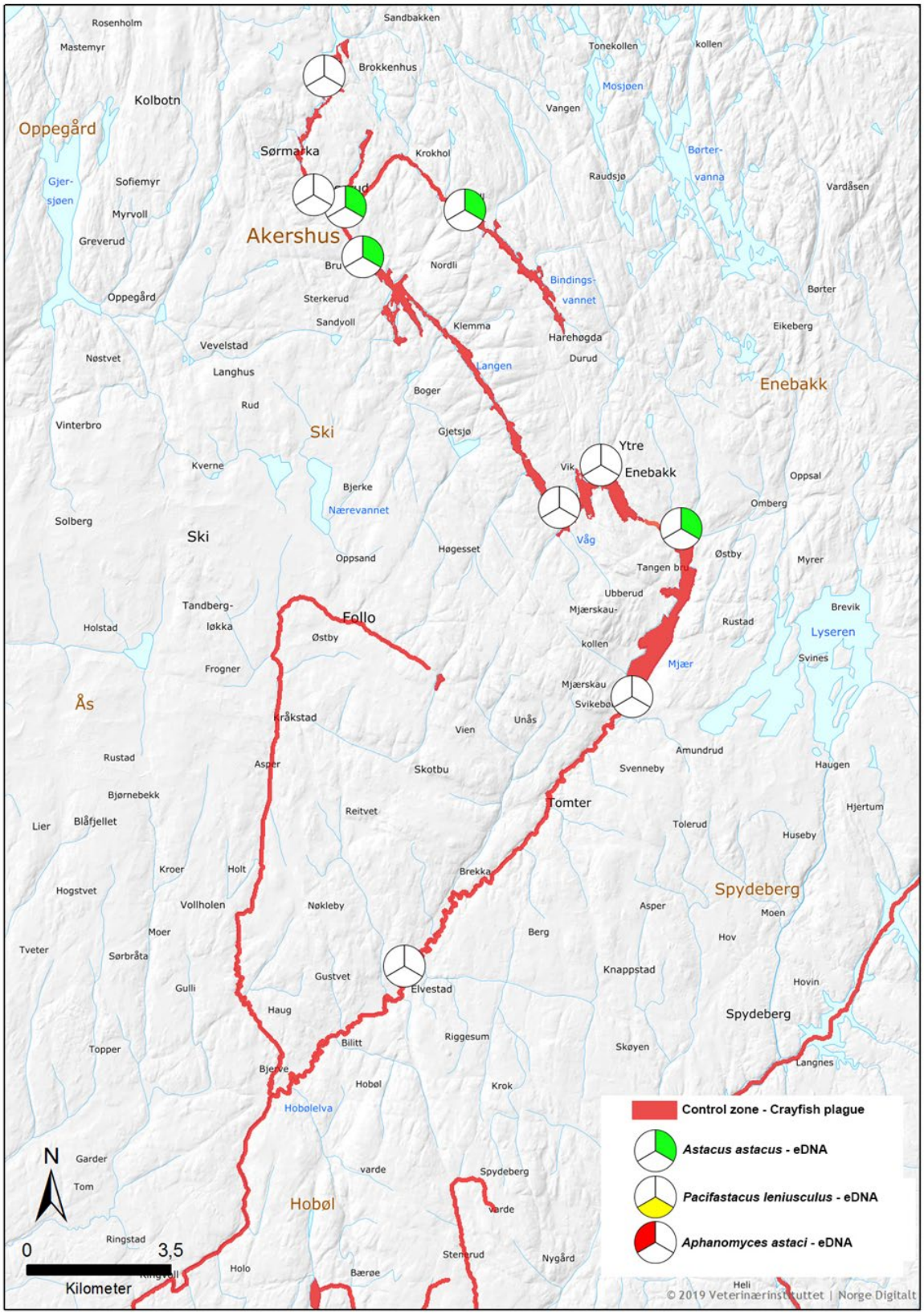

***Figure 6**: Overview map of the surveyed part of the Mosse watercourse. The control area is represented by red colour. The pie chart indicates presence (colour) or absence (white) of A. astaci (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. No eDNA of A. astaci and signal crayfish was detected*

***Figure 7**: Overview map of the Glomma watercourse region and Eidskog municipality. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of A. astaci (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. No eDNA from noble crayfish, signal crayfish or A. astaci were detected in the Glomma watercourse or in the River Buåa. In the Vrangselva watercourse, no eDNA of A. astaci or signal crayfish was detected, while eDNA of noble crayfish was detected at Åbogen to Skotterud. Also, in the River Finnsrudelva, eDNA of noble crayfish was detected but without signs of A. astaci or signal crayfish.*

Conclusion

In the Halden watercourse, combined eDNA monitoring of *A. astaci*, noble crayfish and signal crayfish confirmed that signal crayfish present in Lake Rødenessjøen release detectable, but low concentrations of *A. astaci* to the water. While there were no detection of *A. astaci* in the lower part of River Hølandselva in 2020, the detection in 2019 (10) indicates that there pathogen can still be present in the River in low abundance. There was no sign of *A. astaci* in the northern part of River Hølandselva or in any of the stations in the neighbouring risk areas indicating that the outbreak is limited to the lower part of River Hølandselva. This is also supported with positive samples for noble crayfish eDNA at all stations upstream.

No *A. astaci* positive eDNA samples were observed in the Mosse watercourse in 2020 at the surveyed sites in June and August. While the crayfish plague reached Lake Langen in 2018 (12), detection of noble crayfish eDNA upstream the lake indicates no further spread.

In the Glomma watercourse, no *A. astaci* or signal crayfish eDNA was detected. The status is still highly uncertain, given many years of recurrent crayfish plague detection in cage experiments. However, the results indicate at least that our sampling effort was not sufficient to reveal an eventual infection source in the watercourse. The discovered signal crayfish population downstream Solbergsfoss in Glomma watercourse does not explain the previous outbreaks further upstream, as Solbergsfoss works as a barrier for upstream spread. Positive eDNA results for noble crayfish was found in 2016 (by Opstadåa) and 2019 (by Skarsnes and Vingersnoret), but not in 2017, 2018 or 2020 (6, 10-12).

We found no sign of *A. astaci* in any of the monitored sites in Eidskog municipality. Similar to the results of 2017, 2018 and 2019 (10-12), noble crayfish eDNA was detected at several of the monitored sites in the Vrangselva watercourse and River Finnsrudelva. This supports the view that the crayfish plague has still not yet entered the Norwegian side of these river systems and suggests the presence of live noble crayfish in both systems. Crayfish plague was active in River Finnsrudelva at the border in June 2018 (20-21).

In summary, eDNA from *A. astaci* was not detected anywhere else than from the signal crayfish location in Lake Rødnessjøen. Frequent detections of noble crayfish eDNA within the regulated *A. astaci* control zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggests the presence of vital noble crayfish populations within several of the *A. astaci* regulated and restricted zones. This actualize a coordinated plan for defining criteria for documentation of infection freedom.

Acknowledgements

Both the surveillance programme for *Aphanomyces astaci* in Norway and the national surveillance programme of noble crayfish benefit from the coordinated and simultaneous monitoring of the three target species (crayfish plague, red-listed noble crayfish and black-listed signal crayfish). We thank the Norwegian Food Safety Authority (NFSA) for accepting the

idea of a joint collaborative project between these surveillance programmes. The surveillance programme for *A. astaci* is funded by NFSA, and the national surveillance programme for noble crayfish is funded by the Norwegian Environment Agency (NEA).

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Appendix

Supplementary information to the report "The surveillance programme for Aphanomyces astaci in Norway 2020" - Tables S1 – S5.

***Table S1**: Agreed areas and locations of the "NOK* A. astaci *2020" program. We reserve the right to change and a reallocation of sample localities if new circumstances arise.*

Location	Watercourse[1]/ municipality, county[2]	Location infection status	# water samples (site X samples X visits)
Halden watercourse			**Total samples 48**
Rødenessjøen	HW/Marker, Ø	Control zone	8 (2 x 2 x 2)
Hølandselva	HW/Aurskog-Høland, A	Control zone	8 (2 x 2 x 2)
Fossersjøen	HW/Aurskog-Høland, A	Control zone, outbreak expected	4 (1 x 2 x 2)
Fosserdam overside	HW/Aurskog-Høland, A	Risk zone/control zone border	4 (1 x 2 x 2)
Bjørkelangen	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Lierelva	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Lundsfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Dalstorpsfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Hemnessjøen	Lake/Aurskog-Høland, A	Risk zone	8 (2 x 2 x 2)
Glomma watercourse			**Total samples 20**
Storsjøen	GW/Nord & Sør Odal, H	Control zone	4 (1 x 2 x 2)
Oppstadåa	GW/Sør-Odal, H	Control zone	8 (2 x 2 x 2)
Vingersnoret	GW/ Sør-Odal, H	Control zone	4 (1 x 2 x 2)
Vingersjøen	GW/ Sør-Odal, H	Control zone	4 (1 x 2 x 2)
Eidskog			**Total samples 32**
Buåa	BW/Eidskog, H	Control zone	8 (2 x 2 x 2)
Finnsrudelva	RF/Eidskog, H	Control zone	8 (2 x 2 x 2)
Vrangselva	VW/Eidskog, H	Control zone	16 (4 x 2 x 2)
Mosse watercourse			**Total samples 32**
Hobølelva	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Mjær	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Tangenelva	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Våg badeplassen	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Langen	MV/Enebakk, Ø	Control zone	8 (2 x 2 x 2)
Upstream Langen	MV/Enebakk, Ø	Control zone	16 (4 x 2 x 2)
Total			**140**

[1] HW = Halden watercourse, GW = Glomma watercourse, MW = Mosse-watercourse, BW = Buåa watercourse, RF = River Finnsrudelva, VW = Vrangselva watercourse
[2] Ø = Østfold, A = Akershus, H = Hedmark

***Table S2**: Locations for water sampling in the Halden watercourse area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location[1]	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Lierelva	HA1	R	59°53'8"N 11°34'29"E	5	15	0	3	0	0	2	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	4	20	0	1	0	0	0	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	14	0	3	0	0	1	0
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	9	0	2	0	0	1	0
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	20	0	2	0	0	2	0
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	4	16	0	2	0	0	2	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	4	19	0	2	0	0	2	0
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	5	12	0	2	0	0	3	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	5	14	0	3	0	0	2	0
Hølandselva middle	HA14	C	59°43'13"N 11°29'31"E	5	14	0	3	0	0	2	0
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	5	14	0	2	0	0	3	0
Rødnessjøen Kroksund	HA11	C	59°37'6"N 11°35'5"E	4	20	0	0	0	0	0	0
Rødenessjøen Ysterud	HA12	C	59°29'17"N 11°38'23"E	4	19	2	0	2	0	0	2
Total				57	204	2	25	2	0	20	2

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).
NA = Not available.

***Table S3**: Locations for water sampling in Mosse-watercourse area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Bindingsvann, outlet	MO11	C	59°47'22.1"N 10°57'17.6"E	5	13	0	2	0	0	1	0
Tangentjern, inlet, bridge on Brusagaveien	MO12	C	59°47'18.2"N 10°54'02.9"E	5	18	0	3	0	0	2	0
Sværsvann	MO8	C	59°49'03.2"N 10°53'25.3"E	6	13	0	0	0	0	0	0
Tangentjern, inlet, bridge on Hareveien	MO10	C	59°47'25.7"N 10°53'27.5"E	4	17	0	0	0	0	0	0
Langen, inlet, bridge on Bru-fjellv.	MO9	C	59°46'44.7"N 10°54'38.6"E	5	15	0	2	0	0	0	0
Langen, bridge on Skiveien	MO1	C	59°43'33.3"N 11°00'12.1"E	6	8	0	0	0	0	0	0
Våg	MO2	C	59°44'10.2"N 11°01'14.7"E	4	16	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	4	20	0	1	0	0	0	0
Mjær, outlet	MO6	C	59°41'10.2"N 11°02'27.6"E	4	16	0	0	0	0	0	0
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	5	14	0	0	0	0	0	0
Total				48	149	0	8	0	0	3	0

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S4**: Locations for water sampling in the Glomma region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vingersnoret	GL1	C	60°11'36.3"N 12°01'54.5"E	4	15	0	0	0	0	0	0
North of Vingersnoret	GL2	C	60°11'39.7"N 12°01'41.2"E	5	17	0	0	0	0	0	0
Storsj. Ringåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	4	20	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	16	0	0	0	0	0	0
Glomma, Skarnes	GL10	C	60°15'20.8"N 11°40'49.4"E	4	20	0	0	0	0	0	0
Total				21	88	0	0	0	0	0	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S5**: Locations for water sampling in the Eidskog region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vrangselva, Åbogen	VR1	C	60°06'43.6"N 12°07'01.0"E	4	20	0	2	0	0	2	0
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	5	17	0	1	0	0	2	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	4	14	0	1	0	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	6	13	0	0	0	0	0	0
Finnsrudelva, Finnsrudvegen	FR1	C	59°59'50.7"N 12°19'05.4"E	4	20	0	2	0	0	2	0
Finnsrudelva, Billavegen	FR2	C	59°58'44.9"N 12°20'14.2"E	4	20	0	2	0	0	2	0
Buåa, Eidskog	BU1	C	59°55'31.1"N 11°59'37.0"E	5	13	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	5	18	0	0	0	0	0	0
Total				37	134	0	8	0	0	8	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

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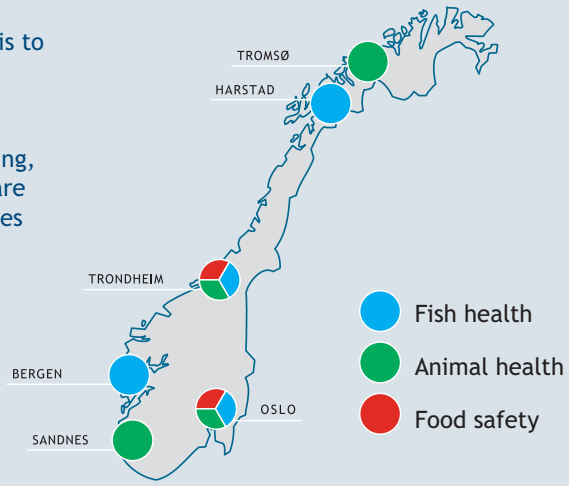

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